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Hunting for clues
Regulatory T cells and colorectal cancer

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Colorectal cancer is a common disease which appears to be influenced by anti-tumor immune responses. Here we discuss our recent study[1] which tracked anti-tumor CD4$^+$ T cell responses before and after surgical resection, highlighting the importance of regulatory T cells (T_{regs}) in controlling these responses, and the influence of the tumor on these specific T-cell populations.

One in three adults die of cancer and the treatment of many solid epithelial malignant tumors remains disappointing. Colorectal cancer (CRC) is one of the most common tumors diagnosed in the developed world. The mainstay of treatment is surgical excision, with adjuvant chemotherapy if the tumor has already spread beyond the confines of the bowel wall or is deemed aggressive following histological examination, for instance, with vascular invasion. There is a huge global effort directed at discovering better treatments for cancer. One potentially fruitful but as yet unfulfilled approach is to utilize the hosts' immune system to generate effective anti-tumor responses leading to control or destruction of the cancer. One of the oldest clinical demonstrations of this approach was the instillation of intravesicular bacillus Calmette-Guerin (BCG) to control bladder cancer. However the oncology field has been filled with scepticism for several reasons, including the failure of the vast majority of clinical immunotherapy trials[2] and indeed, the paucity of demonstrable anti-tumor immune responses in cancer patients often questioned their very existence. One of many reasons for these disappointments may be the activities of a population of natural regulatory T cells (T_{regs}), which appear to inhibit not only auto-reactive T-cell responses, helping prevent autoimmune disease, but also those responses directed at tumors.[3]

Tregs express a series of markers including CD4, CD25 (high levels), and the transcription factor Foxp3. They inhibit the activation and function of other immune cells, including T cells, and are IL-2 dependent for growth and probably function. For functional studies, Foxp3 cannot be utilized as it is a transcription factor and hence requires cell permeabilisation for its detection.

We have set up a program of research to examine how Tregs impinge on patients with colorectal cancer (CRC). Why choose this tumor? First it is a common disease: there are over 250 cases per year in Cardiff (a city of just over 300,000). Second, it has been known for 80 years that patients in whom the resected cancer is markedly infiltrated with immunocytes have a better prognosis.[4] This finding has been confirmed and extended by Galon's group in a widely cited paper in *Science*.[5] Here they showed that patients with an infiltrate of T cells into the tumor had a better prognosis, irrespective of tumor stage. Hence immune responses to the cancer are likely to be important. Third, as over 50% of patients are expected to survive at least one year after resection of the CRC, this enables longitudinal type measurements of T-cell responses to be performed.

In a preliminary study, we found that the depletion from blood of CD4$^+$CD25^{hi} cells unmasked anti-tumor CD4$^+$ T-cell responses, suggesting that a population of Tregs is controlling and indeed preventing effective anti-tumor immune responses.[6] Intuitively, this would appear to be to the detriment of the patient. We performed a follow up study of 62 patients undergoing surgery for CRC. Samples prior and repeatedly post surgery enabled us to track both the Treg population in blood, and measure the effects of surgery ± Treg depletion on anti-tumor immune responses.[1] The presence of CRC in situ was associated with a population of Foxp3 high Tregs, but surgical resection of the cancer led to the levels of Foxp3 in Tregs falling to those of controls (illustrated in **Fig. 1**). These Tregs, measured pre-operatively, suppressed anti-tumor responses, in all patients who had a tumor recurrence at 12 months. Apart from altering the function of Tregs, surgical removal of the tumor also allowed a more robust anti-tumor response to develop, which ex vivo peaked in magnitude at 6 months. Clearly CRCs are potentially immunogenic, but have "evolved" strategies to evade the immune response.

An increase in Tregs has been associated with many tumors, and in turn, implicated in prognosis.[7] Their role in CRC is still unclear as outlined recently in a review by Ladoire and colleagues.[8] This review highlighted that some groups—not all it should be pointed out—have shown a better prognosis in tumors with a higher proportion of Foxp3$^+$ T cells in

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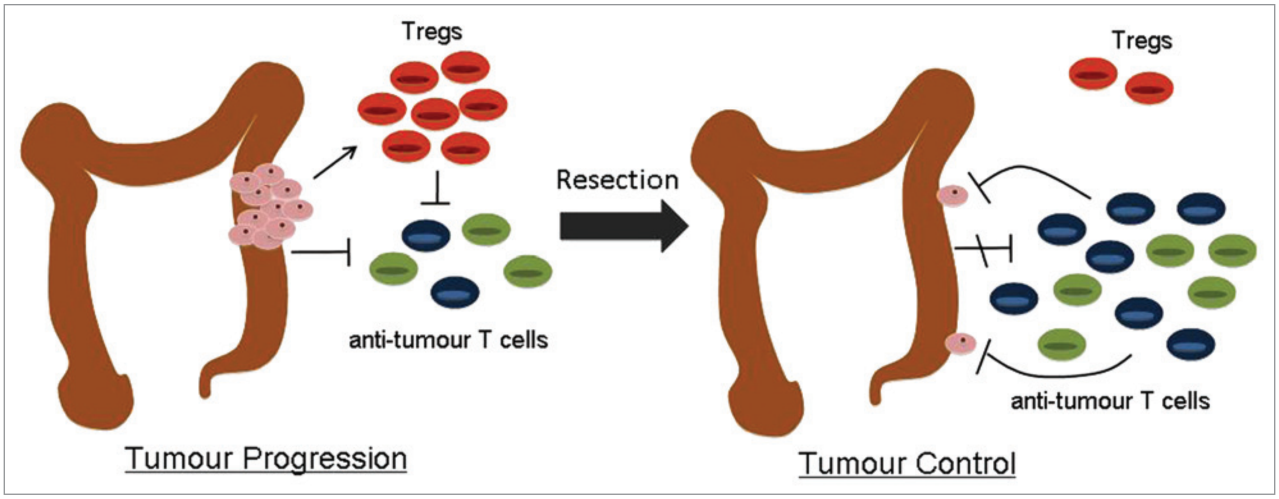

Figure 1. Illustration of the effects on the anti-tumor immune response of surgery. Primary colonic tumors may inhibit immune responses both directly and indirectly by driving T_{regs}. Resection could reverse both these processes allowing potentially useful anti-tumor immune responses to emerge.

the tumor infiltrating lymphocyte (TIL) population. Using immunohistochemistry, we also find a higher ratio of Tregs to $CD3^+$ T cells in early CRCs compared with more advanced cancers that have penetrated the wall of the colon. However it is noteworthy this ratio changes if we use different methods of measuring these cells, such as flow cytometry on purified TILs (unpublished data). Several technical considerations may underpin this difference (e.g., flow cytometry more readily distinguishes between $Foxp3^{hi}$ and $Foxp3^{lo}$ cells) and until the functional significance of TILs is elucidated, these findings will remain difficult to interpret. It may be that the function of Tregs is different in early compared with late tumors. However it is clear that Tregs demonstrate a function in controlling anti-tumor immune responses in CRC, and we feel logically this is most likely to be to the detriment of the patient, particularly after surgery. If it is assumed that anti-cancer T-cell responses are a good thing for the patient, then removing inhibitory influences such as Tregs should be beneficial. Studies seeking to confirm or refute this hypothesis are underway in several laboratories, including our own.

References

1. Betts G, Jones E, Junaid S, El-Shanawany T, Scurr M, Mizen P, et al. Suppression of tumour-specific $CD4^+$ T cells by regulatory T cells is associated with progression of human colorectal cancer. Gut 2012; In press; PMID:22207629.
2. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004; 10:909-15; PMID:15340416; http://dx.doi.org/10.1038/nm1100.
3. Sakaguchi S. Naturally arising Foxp3-expressing $CD25^+CD4^+$ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005; 6:345-52; PMID:15785760; http://dx.doi.org/10.1038/ni1178.
4. McCarty W. Principles of prognosis in cancer. J Am Med Assoc 1931; 96:30-3; http://dx.doi.org/10.1001/jama.1931.02720270032009.
5. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313:1960-4; PMID:17008531; http://dx.doi.org/10.1126/science.1129139.
6. Clarke SL, Betts GJ, Plant A, Wright KL, El-Shanawany TM, Harrop R, et al. $CD4^+CD25^+FOXP3^+$ regulatory T cells suppress anti-tumor immune responses in patients with colorectal cancer. PLoS One 2006; 1:129; PMID:17205133; http://dx.doi.org/10.1371/journal.pone.0000129.
7. Betts GJ, Clarke SL, Richards HE, Godkin AJ, Gallimore AM. Regulating the immune response to tumours. Adv Drug Deliv Rev 2006; 58:948-61; PMID:17070961; http://dx.doi.org/10.1016/j.addr.2006.05.006.
8. Ladoire S, Martin F, Ghiringhelli F. Prognostic role of $FOXP3^+$ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. Cancer Immunol Immunother 2011; 60:909-18; PMID:21644034; http://dx.doi.org/10.1007/s00262-011-1046-y.